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The CHEMISTRY of LIPIDS in HEALTH and DISEASE

A REVIEW OF OUR PRESENT KNOWLEDGE OF LIPIDS; THEIR
CHEMICAL STRUCTURE; THEIR BREAKDOWN AND SYNTHESIS IN
LIVING ORGANISMS; THEIR PLACE IN HUMAN NUTRITION; AND
THEIR ABNORMALITIES OF METABOLISM IN DISEASE

In clear, straightforward style Doctor King deals with
discoveries of the past decade in this wide and ever
expanding field. He writes, not to provide a detailed
guide to the literature or a revision of older theories,
but to convey new ideas--major modern advances.

The role of LIPIDS IN THE DIET is outlined
with an important section on the
ESSENTIAL FATTY ACIDS.

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THE CHEMISTRY OF LIPIDS IN HEALTH AND DISEASE

A review of our present knowledge of lipids; their chemical structure; their breakdown and synthesis in living organisms; their place in human nutrition; and their abnormalities of metabolism in disease.

By

H. K. KING, M.A., PH.D., F.R.I.C.

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AMERICAN LECTURE SERIES*

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New York City

PREFACE

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LIPIDS used to be a dull subject for any save a few chemical specialists. Their dietary role did not appear to extend beyond that of an important though inessential energy-source; the biochemists knew but little about their metabolic pathways; and they held but little interest for either the general medical practitioner or the specialist pathologist. To-day the chemistry of living organisms is making rapid advances. The second half of the twentieth century has already yielded more knowledge than the first about the part played by lipids in the processes of life. This new knowledge has an interest well beyond academic circles. One of the most urgent medical problems of to-day is a condition in which malfunction of fat metabolism probably plays a critical role.

A problem faces the medical man—whether he be a student, in practice, or engaged in research—who wishes to understand this new situation. Excellent specialist monographs are available on most aspects of lipid biochemistry but he will probably lack both the basic knowledge and the time to study them. I have written this book to help such people. It tries to cover all the relevant parts of the lipid field, except for the steroid hormones: these are being treated in another monograph in this series. It makes no attempt to be a comprehensive or detailed guide to the literature. The bibliographies cite only a few reviews, monographs, and papers which may give a lead to the reader who wishes to investigate the original literature himself.

I have tried to outline the rules, rather than catalogue the exceptions; to give clear pictures which the reader can accept or reject on his own judgment, rather than be scrupulously fair to all points of view in unresolved controversies. My aim is not to influence the reader's judgment, but to give him a background against which he can read and judge current literature, and himself pass judgment on present and future concepts.

I owe a great debt to friends and colleagues—especially to Professor Goodwin, Dr. Glover, Dr. Pitt, and Dr. Wright—for their critical assessment of various sections of this book. Few authors are fortunate enough to have the friendly help of such a team of experts. The errors which remain are mine!

H. K. KING

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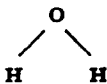
**THE CHEMISTRY OF LIPIDS
IN HEALTH AND DISEASE**

Chapter 1

LIPIDS AND WATER

THIS is a book about the "living chemistry" of lipids. Life is essentially something which goes on in the presence of water. Lipids do not readily enter into intimate relationship with water; they are, we say, characteristically water-insoluble. We cannot get far in understanding their relation to life unless we first master the nature of their relationship with water. This involves two parties—the lipid, and water. We thus need, first, to know something about the structure and properties of water. These seem familiar enough; its formula is H_2O , its melting-point 0° and its boiling-point 100° on the centigrade scale, and so on. But the accuracy of the first of these three statements is open to question, and the second two, though apparently commonplace, reveal a remarkable state of affairs. Why should water boil at 100° ? Its analogue, hydrogen sulphide, one would expect to have a much *higher* boiling point; but it boils at -62° . Why?

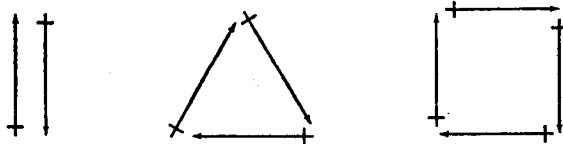
Oxygen is potentially a tetravalent element; though this becomes apparent only rarely (in the "oxonium" compounds). It shares this tetravalency with carbon and other elements of the first round of the Periodic Table. Even when behaving as a divalent element its valencies will—like those of carbon—be separated by the tetrahedral angle of 109° . The water molecule, usually written $H-O-H$, would be better represented as



The oxygen

atom exerts a powerful electron-attracting force and there will tend to be a negative charge around it, with corresponding positive charges around the hydrogens. The water molecule will thus be a "dipole," one end being electrically negative, the other positive. This is often written as an arrow, thus: \rightarrow the head repre-

senting the negative end, the tail, with its cross (+) sign, the positive region. When a mass of such molecules find themselves together, they will tend to line up together, in pairs, triangles, or groups of four, with mutual neutralisation of their opposite charges:




Water molecules thus have great power of self-attraction. In bulk they are very hard to separate, because separation will require the pulling apart of opposite charges against great electrostatic force. It will require more heat—that is, more violent molecular agitation—to separate water molecules than would be needed for similar molecules lacking this special structure. Water therefore has a relatively high boiling-point in contrast with, for example, hydrogen sulphide or its neighbours in the periodic table—ammonia and hydrogen fluoride. Hydrogen sulphide is its nearest analogue; but sulphur is in the second round of the table, and its full valency number is six. The six valencies are arranged along the three dimensions of space: so the H_2S molecule must either be arranged in a straight-line or bent at a right-angle. It is known to be a linear molecule and hence, even if there is an inequality of charge between the hydrogens and the sulphur, the molecule as a whole will be electrostatically symmetrical and will show none of the peculiar properties of water.

We can now see the basis for much of the curious physical behaviour of water. The "association" of its molecules accounts for the very high latent heat of vaporisation (about ten times that of a simple hydrocarbon): much energy is needed to separate molecules so closely bound together. Its specific heat is likewise high, because on heating water much energy is needed to break down complexes such as H_4O_2 , H_6O_3 , etc. into simpler units and eventually into the monomolecular form. The anomalous contraction of water on warming from 0°C to $+4^\circ\text{C}$ arises from the same cause—change in the type of molecular species causes a contraction in volume which more than masks the normal expansion of a heated liquid.

It is also rearrangement of these associated units which causes ice to contract on melting. Most solids expand when changing to the liquid form. Two other properties call for special mention. One is the very high dielectric constant—the value is among the highest recorded. This permits it to loosen the powerful electrostatic attraction between the ions of salts. The ions may then separate; once this has happened, the ionic charge will pull in water molecules and surround the ion with a layer of oriented dipoles. The ion has thus become “associated” with the water, a process known as “solvation.” The ion now has a “surface” of water, and can move freely among water molecules. This is the mechanism by which an ionisable salt dissolves in water. It is beyond the scope of this book to explain how this is brought about—a very good account is given by Edsall and Wayman (Ref. 1). We need only note that it is its dipole characteristic which makes water such a ready solvent for most inorganic salts and for many organic materials.

The other important property is the high surface-tension of water. This is sometimes cited as evidence of an “internal pressure” and provides visible evidence of the powerful intermolecular attractions between water molecules. This has another consequence. Water molecules draw together—and that means that they will tend to squeeze out the stranger. Hydrocarbons are insoluble in water, not because of any repulsive force between the two, but because the attraction of the water molecules for each other is so powerful that the inert hydrocarbon molecules are pushed together on one side. Leaving aside the question of inorganic salts, it follows that water will mix readily only with those substances with which it has something in common—and that something is usually an asymmetry of electric charge. Broadly speaking, this asymmetry will be found mainly among two classes of organic compounds—ions and hydroxyl compounds. The latter will obviously have a

H

similar arrangement to water, $\text{R}-\text{O}$ , with a “bent” molecule, and will be able to take part in the groupings with water molecules to a greater or lesser extent. Indeed, the lower aliphatic alcohols share many of the peculiar properties of water though to a much lesser degree. When we come to the ethers these properties have

almost vanished. Ordinarily diethyl ether has a much lower boiling-point than butanol, which has the same molecular formula; but is still well above the boiling-point of the corresponding hydrocarbon; and ether is only slightly soluble in water.

We find a different situation when we examine molecules which have a dual character, such as combinations of a long hydrocarbon chain with a hydroxyl group or an ionising radical. Such compounds are frequently met among the lipids. Their behaviour is

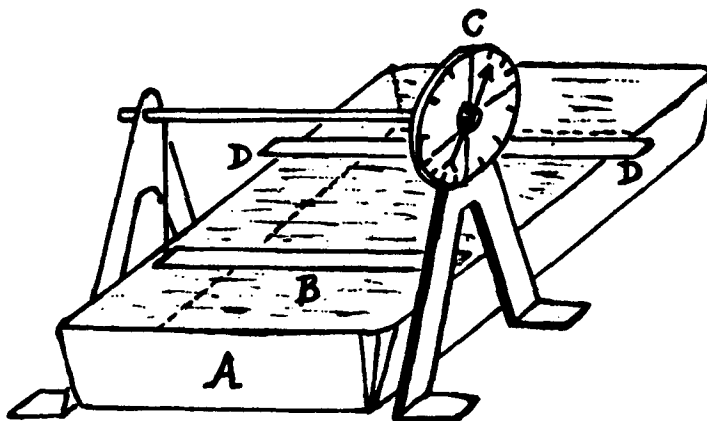


FIG. 1. Apparatus for study of behaviour of fatty acids at water/air interfaces.

most interesting. It was first investigated by Langmuir with the aid of the apparatus named after him (Fig. 1). This consists of a shallow rectangular trough (A) filled to the brim with water. At one end a thin strip of mica rests on the surface of the water (B) suspended by a wire stirrup attached to a torsion head (C). At the other end of the trough, a movable strip of glass (DD) rests on the sides of the trough and makes contact with the surface of the water. A rectangular area of water is thus enclosed by the glass strip DD, the sides of the trough, and the freely-suspended mica strip B. The latter does not touch the sides of the trough, but connects with them by means of a loose cotton thread lying on the surface of the water at each side: this allows free movement of the mica strip and at the same time effectively seals off the enclosed area of water.

A small quantity of (say) stearic acid is now distributed over the surface of the enclosed area of water. This is best done by dissolving the stearic acid in ether, and dropping a suitable amount of the solution onto the water surface: the ether evaporates and leaves an invisible film of the fatty acid. Immediately the mica strip B moves,¹ as if a pressure is being exerted on it by the fatty acid; and this pressure can be measured by bringing it back to its original position by means of the torsion head. If the glass strip DD at the far end of the trough is now moved over the surface of the water towards the mica, the pressure increases. At first the pressure—which is very weak—follows approximately the relation:

$$\text{Pressure} \times \text{Area} = \text{Constant} \times T$$

where T is the absolute temperature. But on closer approach of the glass strip, i.e., on compression of the film of fatty acid—a point is reached where further slight reduction in area causes a disproportionate increase in pressure. At the same time, a film of fat can be seen on the surface of the water. Further compression causes visible buckling of this film, and the pressure/area relationship becomes irregular. The whole sequence can be seen in Fig. 2.

What has been happening? The stearic acid consists of an ionising, i.e., water-soluble carboxyl group—which we can conveniently call the “head” of the molecule—and a long hydrocarbon “tail” which is water-insoluble and sometimes described as water-repellent—though the latter term is hardly correct; we have seen the situation is rather that the water-molecules have a very great attraction for each other but none for the hydrocarbon tail. The “head” will thus tend to go down into the water, and the “tail” will stay on the surface, thus:



The stearic acid molecules (if there are not too many of them) will move about freely and bump into each other and also into the sides of the containing vessel. The latter effect will give rise to the pressure recorded by the torsion head controlling the mica strip. We have, in fact, what might be described as “a two-dimensional gas,” and, corresponding to Boyles’s law,

¹ To the left, in the diagram.

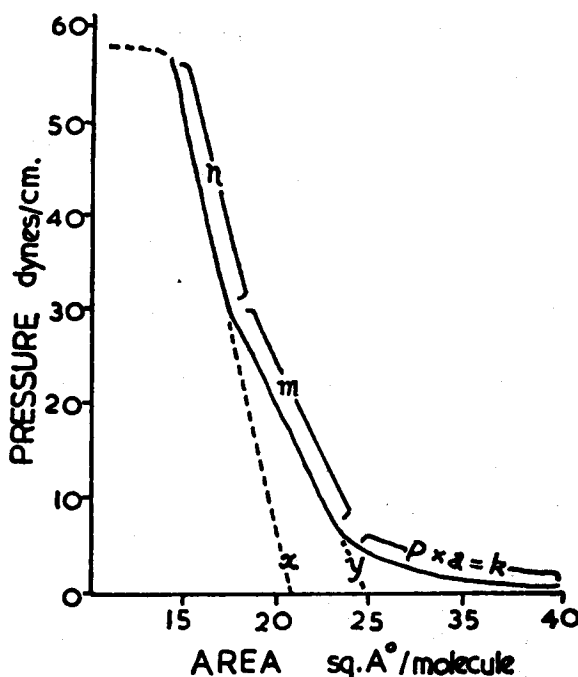


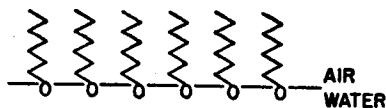
FIG. 2. Compression of a monomolecular film of stearic acid.

$$\text{Pressure} \times \text{Volume} = \text{Constant}$$

we have (as found experimentally)

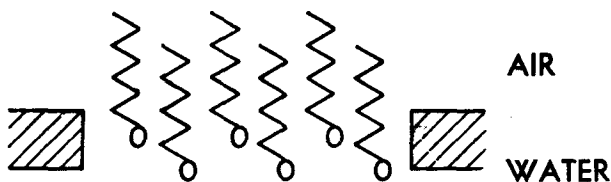
$$\text{Pressure} \times \text{Area} = \text{Constant}$$

On compression, a point will be reached, sooner or later, where the molecules will become crowded and the simple law no longer applicable. We may even "liquefy" the film. In the case of stearic acid however the result will be to force the molecules to stand on their heads, so to speak:



The film (or "monolayer," as it is called) will now evidently resist further compression, and pressure rises steeply if the area is reduced;

this accounts for the slope m in Fig. 2. At even higher pressures the molecules may be forced to close their ranks still further.

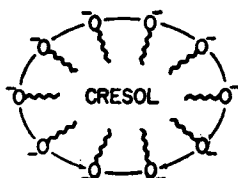


giving rise to the steeper slope n . By extrapolating these two slopes to zero pressure, we can obtain the values for the areas of cross-section of the carboxyl "head" (y) and the hydrocarbon "tail" (x).

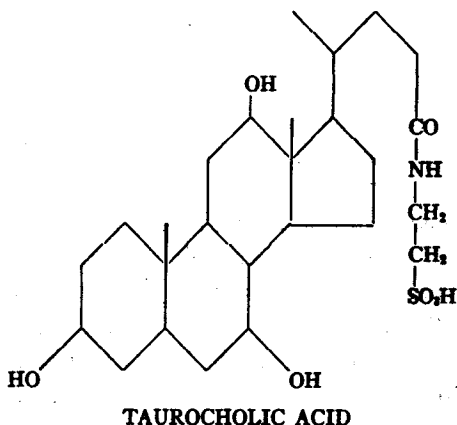
We can also interpret the changes in pressure in somewhat different terms. The apparent pressure exerted by the fatty-acid film might be regarded as a *lowering* of surface **tension**. The mica strip is being *pulled* towards the left (in Fig. 1) rather than *pushed* from the right, because to the left of it lies a surface of water which, by virtue of its intermolecular attractions has a very high surface tension, whilst to the right lies a surface consisting—wholly or partly—of fatty acid, which has a low surface tension. Which is formally the more accurate interpretation is a matter for the physical chemists; all we are concerned with is the property of fatty acids to collect at an interface—such as an air-water surface—and form a monolayer endowing the water surface with properties quite different from those of the pure liquid. A striking example of how different these properties may be has been shown by the fact that cetyl alcohol (which, by virtue its —OH group can behave in a similar way) will reduce surface evaporation by more than 50% if it is spread in a layer one molecule deep on the surface of a reservoir. This property may prove of great value in conservation of water in dry areas.

These phenomena may appear at interfaces other than that of air-water. This may result, for example, in substances not normally soluble in water being dispersed so as to give the appearance of true solution. A familiar example is the well-known disinfectant lysol. The active agent is a mixture of the isomers of cresol, which have only a slight solubility in water. Lysol however contains soap; and the soap molecules can array themselves round a sphere of cresol,

with "tails" in towards the cresol and the ionised "heads" outwards, thus:

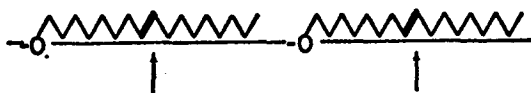


We have now provided our cresol with an outer coating of ionisable groups, and rendered it soluble in water. The complex of cresol and fatty anion is referred to as a "micelle." It is the property of dispersing insoluble materials in this way which accounts for the action of soap as a cleansing agent. Such substances are often known as "detergents." Soap is not a particularly efficient detergent, since its carboxyl group is only relatively poorly ionised (save at alkaline pH). If we replace the weakly acidic carboxyl group by a strongly ionised group (e.g., $-\text{SO}_3$) we obtain a much more efficient detergent action. Many of the common domestic detergents are essentially hydrocarbon chains carrying a sulphate group. A strongly basic group would be equally effective; but for various practical reasons these find relatively little application as detergents. Sulphonic acids are not commonly found in nature; but one of the most familiar of natural detergents belongs to this class.



Taurocholic acid has, in addition to the sulphonate group, three —OH groups; its basic structure is not a hydrocarbon chain, but the relatively flat steroid nucleus. It is thus admirably fitted to attach itself and to cover surfaces. Its action in emulsifying fats prior to digestion is well known. The ability of lipid monolayers to act as barriers in separating potentially reactive metabolites is of much greater biological significance than their detergent action.

Oxidation of oleic acid by potassium permanganate was one of the first reactions shown to be controlled in this way. If a small amount of oleic acid is spread on the surface of water, the molecules can move about freely and the double-bond will be available to any reactive reagent dissolved in the water:



But if the film is compressed, then the double-bond will be forced away from the surface. Experiment shows that simple compression of the film will arrest the oxidation of the double-bond. This is of vital importance. It provides a clue to how different chemical reactions may be “kept apart” in a cell which is composed mainly of an aqueous gel. Such barriers could be crossed only by a substance possessing some degree of lipid-solubility. When a homologous series of compounds with biological activity is studied, it is often found that the relative activity of the various compounds is directly related to their lipid solubility.

What compounds are likely to be used in the formation of these monolayers? The fatty acids themselves are hardly suitable since they are too weakly ionised at physiological pH. Nor do they occur free in the cell to any great extent. Proteins have possibilities; the amino-acids are all of the same steric (L) configuration, so the side-chains (which may bear ionisable groups) all lie on the same side of the main chain. A protein may therefore present two surfaces of differing properties, one hydrophilic, one lipophilic. Some proteins can indeed be spread on the surface of water to form a monolayer. But among the lipids themselves the phosphatides are the best suited to form monolayers at interfaces. With strong (phosphoric) acid radicals and strong basic groups such as the quaternary am-